

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11) Publication number:

0 484 936 A1

(12)

EUROPEAN PATENT APPLICATION(21) Application number: **91118982.7**(22) Date of filing: **07.11.91**(51) Int. Cl.⁵: **A61K 9/06, A61K 31/44,
A61K 31/445, A61K 31/55,
A61K 47/26**(30) Priority: **08.11.90 JP 304839/90
07.10.91 JP 259358/91
07.03.91 GB 9104834**(43) Date of publication of application:
13.05.92 Bulletin 92/20(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE(71) Applicant: **FUJISAWA PHARMACEUTICAL CO.,
LTD.****4-7, Doshomachi 3-chome Chuo-ku
Osaka-shi Osaka 541(JP)**(72) Inventor: **Asakura, Sotoo
6-10-18, Oe-nishishinbayashi-cho,
Nishikyo-ku****Kyoto-shi, Kyoto(JP)
Inventor: Koyama, Yasuto
2-17-1002, Chuo 6-chome
Itami-shi, Hyogo(JP)****Inventor: Kiyota, Youhei
2-10-1-201, Sumiyoshi
Ikeda-shi, Osaka(JP)****Inventor: Akashi, Kiyoko
42, Nakasuji 3-chome
Takarazuka-shi, Hyogo(JP)****Inventor: Kagayama, Akira
13-7, Mayumiminami 1-chome
Ikoma-shi, Nara(JP)****Inventor: Murakami, Yoshio
8-8-7, Suzurandahigashimachi, Kita-ku
Kobe-shi, Hyogo(JP)****Inventor: Nakate, Toshiomi
1-4-3-511, Higashikawasaki-cho, Chuo-ku
Kobe-shi, Hyogo(JP)**(74) Representative: **Türk, Gille, Hrabal
Brucknerstrasse 20
W-4000 Düsseldorf 13(DE)**(54) **Suspensions containing tricyclic compounds.**

(57) A suspendible composition comprising a tricyclic compound such as FK 506 substance which is 17-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone, or the like, and a pharmaceutically acceptable surfactant, which can be used as an orally administable agent or eye drops and is useful for treating various diseases.

BACKGROUND OF THE INVENTION

Field of the Invention

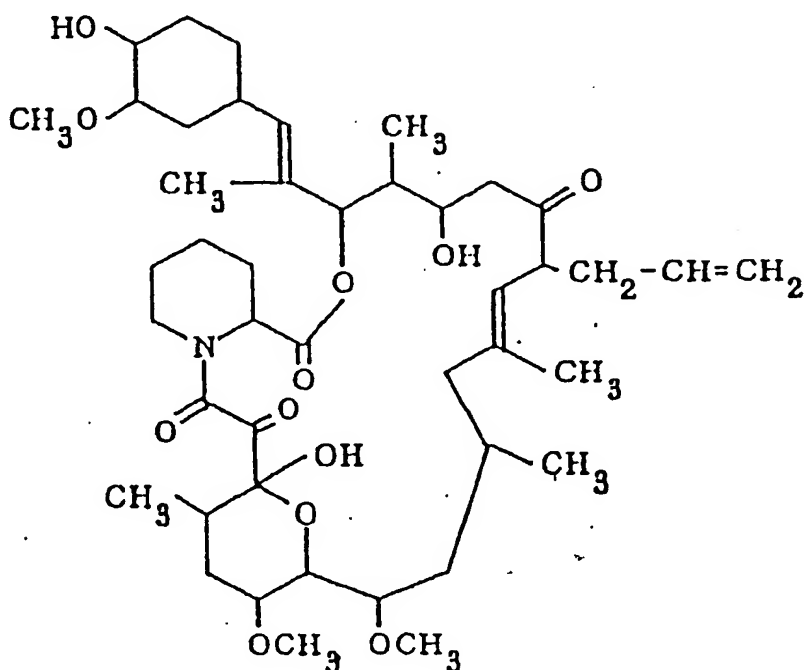
The present invention relates to a suspendible composition comprising fine particles of a tricyclic compound or its pharmaceutically acceptable salt and a pharmaceutically acceptable surfactant. The suspendible composition according to the present invention is useful as an orally administrable agent or eye drops.

Prior Art

Tricyclic compounds as shown by the below-mentioned general formula (I) and pharmaceutically acceptable salts thereof used in this invention have been known to possess excellent pharmacological activities such as immunosuppressive activity and antimicrobial activity, thereby useful for treating and/or preventing rejection against organs or tissue transplantation, graft versus host reaction, various autoimmune diseases and infectious diseases (Japanese Laid-Open Patent Application No. 61(1986)-148181, EP-A-0323042).

Particularly, FR 900506 substance which equals to FK 506 substance, FR 900520 substance, FR 900523 substance and FR 900525 substance are produced by fermenting Genus *Streptomyces*, in particular, *Streptomyces tsukubaensis* No. 9993 (FERM BP-927) or *Streptomyces hygroscopicus* subsp. *yakushimaensis* No. 7238 (FERM BP-928). Particularly, FK 506 substance represented by the following formula possesses excellent immunosuppressive activity, thus useful for treating and/or preventing rejection against organs transplantation and diseases in the ophthalmology.

FK 506 substance:



Chemical name: 17-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone.

5 Injections or capsules for oral use of the tricyclic compound (I) have already been studied. The capsules are generally desirable preparation than the injections from the viewpoint of convenience, but would be difficult to slightly adjust an administration amount of active ingredient and also is not so easy to use for children. Thus, there is a demand for the development of liquid formulations which are excellent in absorption ability when administered orally.

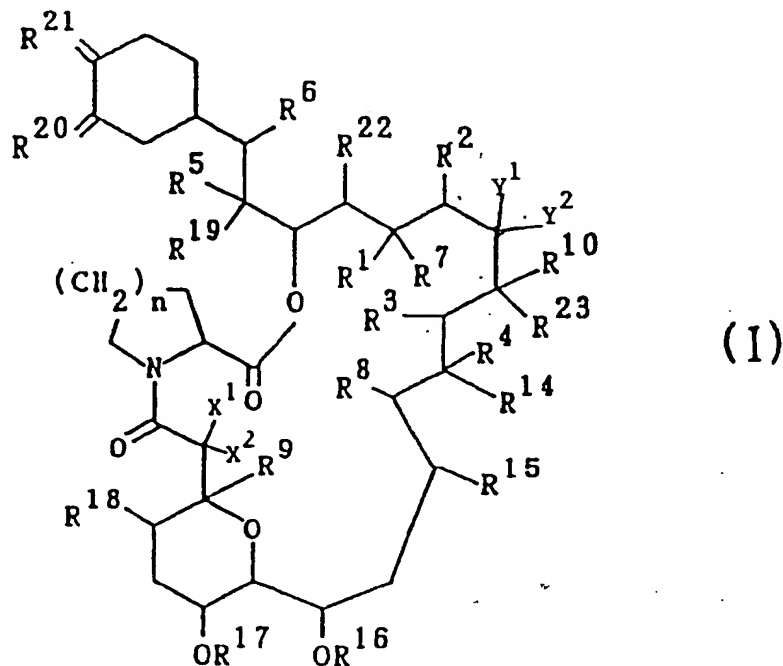
10 When the tricyclic compound (I) of the present invention is employed in ophthalmology, a concern is caused about the appearance of systemic adverse effect upon its oral administration, intramuscular injection or intravenous injection. Accordingly, eye drops for local administration are preferable in ophthalmology as a formulation capable of reducing the appearance of the adverse effect and attaining the desired object with less administration amount.

15 The tricyclic compound (I) of the present invention is well-soluble in organic solvents and fats and fatty oils, whereas it is very slightly soluble in water. Therefore, the tricyclic compound (I) is made into oily eye drops or ophthalmic ointments, similar to the general slightly soluble agents, to be used in ophthalmology. However, the oily eye drops or ophthalmic ointments of the compound (I) are disadvantageous in that the tricyclic compound (I) is remarkably poor in penetration into ocular tissues and additionally are accompanied with evanescent paropsia or unpleasantness, thus impractical.

20 Consequently, there has arisen a strong demand for the development of eye drops comprising the tricyclic compound (I) and having improvements in penetration into ocular tissues and in the aforesaid drawbacks.

SUMMARY OF THE INVENTION

25 The present invention provides a suspendible composition comprising fine particles of a tricyclic compound represented by the following formula (I):



30 wherein each of adjacent pairs of R¹ and R², R³ and R⁴ or R⁵ and R⁶ independently

(a) is two adjacent hydrogen atoms, or

(b) may form another bond formed between the carbon atoms to which they are attached, and further, R² is an alkyl group;

35 R⁷ is a hydrogen atom, a hydroxy group, a protected hydroxy group or an alkoxy group, or an oxo group together with R¹;

R⁸ and R⁹ each is independently a hydrogen atom or a hydroxy group;

R¹⁰ is a hydrogen atom, an alkyl group, an alkyl group substituted by one or more hydroxy groups, an

alkenyl group, an alkenyl group substituted by one or more hydroxy groups or an alkyl group substituted by an oxo group;

X¹ is a hydrogen atom or a hydroxy group;

X² is a hydrogen atom; or

5 X¹ and X² may together represent an oxo group or -CH₂O-;

Y¹ is a hydrogen atom or a hydroxy group;

Y² is a hydrogen atom; or

Y¹ and Y² may together represent an oxo group, N-NR¹¹R¹² or N-OR¹³;

R¹¹ and R¹² each is independently a hydrogen atom, an alkyl group, an aryl group or a tosyl group;

10 R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²² and R²³ each is independently a hydrogen atom or an alkyl group;

R²⁰ and R²¹ each is an oxo group or independently (R²⁰a and a hydrogen atom) or (R²¹a and a hydrogen atom) in which R²⁰a and R²¹a each independently is a hydroxy group, an alkoxy group or a group represented by the formula -OCH₂OCH₂CH₂OCH₃, or R²¹a is a protected hydroxy group, or R²⁰a and R²¹a may together represent an oxygen atom in an epoxide ring;

15 n is an integer of 1, 2 or 3;

in addition to their above definitions, four of Y¹, Y², R¹⁰ and R²³, together with the carbon atoms to which they are attached, may represent a saturated or unsaturated 5- or 6-membered nitrogen, sulfur and/or oxygen containing heterocyclic ring optionally substituted by one or more groups selected from the group consisting of an alkyl, a hydroxy, an alkyl substituted by one or more hydroxy, an alkoxy, benzyl and a

20 group of the formula -CH₂Se(C₆H₅);

or a pharmaceutically acceptable salt thereof, said fine particles of the tricyclic compound and its pharmaceutically acceptable salt having the average size of 5 μm or below; and a pharmaceutically acceptable surfactant, and optionally an aqueous medium.

The present invention also provides a process for preparing the above suspendible composition.

PREFERRED EMBODIMENTS OF THE INVENTION

Preferable examples and explanations for various definitions included in the scope of this invention will be detailed hereinbelow.

30 The term "suspendible composition" means a composition capable of suspending in an aqueous medium. The suspendible composition includes the one already suspended in an aqueous medium and the one which will be suspended therein on use.

The term "lower" as used in this specification means, unless otherwise indicated, a group having 1 to 6 carbon atoms.

35 Preferable examples of the "alkyl groups" are a straight or branched chain aliphatic hydrocarbon residue, for example, a lower alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, neopentyl, hexyl and the like.

Preferable examples of the "alkenyl groups" are a straight or branched chain aliphatic hydrocarbon residue having one double-bond, for example, a lower alkenyl group such as vinyl, propenyl, butenyl, 40 methylpropenyl, pentenyl, hexenyl and the like.

Examples of the "aryl groups" include phenyl, tolyl, xylyl, cumenyl, mesityl, naphthyl and the like.

Preferable protective groups in the "protected hydroxy groups" are 1-(lower alkylthio)(lower)alkyl group such as a lower alkylthiomethyl group (e.g., methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl, butylthiomethyl, isobutylthiomethyl, hexylthiomethyl, etc.), more preferably C₁₋₄ alkylthiomethyl 45 group, most preferably methylthiomethyl group; trisubstituted silyl group such as a tri(lower)alkylsilyl (e.g., trimethylsilyl, triethylsilyl, tributylsilyl, tert-butyltrimethylsilyl, tri-tert-butylsilyl, etc.) or lower alkyl-diarylsilyl (e.g., methyldiphenylsilyl, ethyldiphenylsilyl, propyldiphenylsilyl, tert-butyl-diphenylsilyl, etc.), more preferably tri(C₁₋₄)alkylsilyl group and C₁₋₄ alkyl-diphenylsilyl group, most preferably tert-butyl-dimethylsilyl group and tert-butyl-diphenylsilyl group; or an acyl group such as an aliphatic or aromatic acyl group derived from 50 a carboxylic acid, sulfonic acid and carbamic acid, or an aliphatic acyl group substituted by an aromatic group.

Examples of the aliphatic acyl groups are a lower alkanoyl group optionally having one or more suitable substituents such as carboxy, e.g., formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, carboxyacetyl, carboxypropionyl, carboxybutyryl, carboxyhexanoyl, etc.; a cyclo(lower)alkoxy- 55 (lower)alkanoyl group optionally having one or more suitable substituents such as lower alkyl, e.g., cyclopropyloxyacetyl, cyclobutyloxypropionyl, cycloheptyloxybutyryl, menthyloxyacetyl, menthyloxypropionyl, menthyloxybutyryl, menthyloxy-pentanoyl, menthyloxyhexanoyl, etc.; a camphorsulfonyl group or a lower alkylcarbamoyl group having one or more substituents such as carboxy or protected carboxy, for

example, carboxy(lower)alkylcarbamoyl group (e.g., carboxymethylcarbamoyl, carboxyethylcarbamoyl, carboxypropylcarbamoyl, carboxybutylcarbamoyl, carboxypentylcarbamoyl, carboxyhexylcarbamoyl, etc.), protected carboxy(lower)alkylcarbamoyl group such as tri(lower)alkylsilyl(lower)alkoxycarbonyl(lower)-alkylcarbamoyl group (e.g., trimethylsilylmethoxycarbonylethylcarbamoyl, trimethylsilylethoxycarbonylpropylcarbamoyl, triethylsilylethoxycarbonylpropylcarbamoyl, tertiary butyldimethylsilylethoxycarbonylpropylcarbamoyl, trimethylsilylpropoxycarbonylbutylcarbamoyl, etc.) and so on.

Examples of the aromatic acyl groups are an aroyl group optionally having one or more suitable substituents such as nitro, e.g., benzoyl, toluoyl, xyloyl, naphthoyl, nitrobenzoyl, dinitrobenzoyl, nitronaphthoyl etc.; or an arenesulfonyl group optionally having suitable substituents such as halogen, e.g., benzenesulfonyl, toluenesulfonyl, xylenesulfonyl, naphthalenesulfonyl, fluorobenzenesulfonyl, chlorobenzenesulfonyl, bromobenzenesulfonyl, iodobenzenesulfonyl, etc.

Examples of the aliphatic acyl groups substituted by aromatic group include ar(lower)alkanoyl group optionally having one or more substituents such as lower alkoxy or trihalo(lower)alkyl, e.g., phenylacetyl, phenylpropionyl, phenylbutyryl, 2-trifluoromethyl-2-methoxy-2-phenylacetyl, 2-ethyl-2-trifluoromethyl-2-phenylacetyl, 2-trifluoromethyl-2-propoxy-2-phenylacetyl, etc.

More preferable acyl groups among the aforesaid acyl groups are C₁₋₄ alkanoyl group optionally having carboxy, cyclo(C₅₋₆)alkoxy(C₁₋₄)alkanoyl group having two (C₁₋₄)alkyl at the cycloalkyl moiety, camphorsulfonyl group, carboxy(C₁₋₄)alkylcarbamoyl group, tri(C₁₋₄)alkylsilyl(C₁₋₄)alkoxycarbonyl(C₁₋₄)-alkylcarbamoyl group, benzoyl group optionally having one or two nitro groups, benzenesulfonyl group having halogen or phenyl(C₁₋₄)alkanoyl group having C₁₋₄alkoxy and trihalo(C₁₋₄)alkyl group. Among these, the most preferable ones are acetyl, carboxypropionyl, menthyloxyacetyl, camphorsulfonyl, benzoyl, nitrobenzoyl, dinitrobenzoyl, iodobenzenesulfonyl and 2-trifluoromethyl-2-methoxy-2-phenylacetyl.

Examples of the "heterocyclic groups" in the saturated or unsaturated 5- or 6-membered nitrogen, sulfur and/or oxygen containing ring include a pyrrolyl group or a tetrahydrofuryl group.

The pharmaceutically acceptable salts of the compounds (I) include conventional non-toxic and pharmaceutically acceptable salts such as the salts with inorganic or organic bases, specifically, an alkaline metal salt such as sodium salt or potassium salt, an alkaline earth metal salt such as calcium salt or magnesium salt, an ammonium salt or an amine salt such as triethylamine salt or N-benzyl-N-methylamine salt.

With respect to the tricyclic compound (I) of this invention, it is to be understood that there may be one or more conformers or stereoisomers such as optical and geometrical isomers due to asymmetric carbon atom(s) and double bond(s), and such isomers are also included within a scope of this invention.

According to the present invention, the tricyclic compound (I) or its salt is required to be used as fine particles having the average size of 5 μm or below. The particles may be either of crystalline or amorphous ones. The fine particles preferably have an average size of 3 μm or below, or 2 μm or below, for example, 0.9 μm , 0.8 μm or 0.7 μm . Such average size is significantly very small compared to the particle size of 75 μm or below which is preferable to use suspension type of eye drops (refer to the explanation of eye drops in Japanese Pharmacopoeia) or general particle size of 10 μm or below.

The fine particles of the tricyclic compound (I) or its pharmaceutically acceptable salt used in the present invention can be prepared by the following process.

It comprises a step of dissolving the tricyclic compound (I) (including its salt hereinbelow) in a solvent and a step of precipitation from the solution obtained by the previous step. Examples of the solvents used for dissolving the compound (I) include organic solvents capable of dissolving the compound (I), especially organic solvents having a large solubility toward the compound (I) and compatibility with water, for example, alcohols such as ethanol, acetone, acetonitrile, dioxane or the like. The amount of the solvent to be used is preferably 3 to 10 times that of the compound (I). A surfactant, such as nonionic surfactant should be added to the solution. The surfactant may be added to the solvent before dissolving the compound (I) therein. Otherwise, an aqueous surfactant solution may be prepared in advance.

Subsequently, water is added to the obtained solution which is then vigorously stirred to precipitate crystals. The mixture including the crystals is subjected to aging, concentration and micronization (e.g., ultrasonification) if necessary. The mixture mentioned above is finally passed through a filter having a predetermined mesh (e.g., millipore filter of 0.45 μm) to obtain a fine particle (which generally comprises fine crystals) having a desired particle size. Alternatively, the mixture is lyophilized after the micronization, thereby obtaining a lyophilized product of fine particles having a desired particle size. The lyophilized product includes the surfactant as well.

The preparation of the above-mentioned fine particles or lyophilized product may be performed after the addition of an anticoagulating agent, antifoaming agent, isotonic agent, buffer agent, preservatives, thickener or the like in a suitable amount.

Preferable pharmaceutically acceptable surfactants used for this invention are nonionic surfactants. Examples of the nonionic surfactant are as follows.

A. Ether type

Polyoxyethylene alkyl ethers

- 10 Polyoxyethylene polyoxypropylene block polymer
Polyoxyethylene polyoxypropylene alkyl ethers

B. Ether-ester type

- 15 Polyoxyethylene glycerine fatty acid esters
Polyoxyethylene sorbitan fatty acid esters such as
polysolvate 80 (monooleic polyoxyethylene sorbitan)
polyoxyethylene sorbitol fatty acid esters

20 C. Ester type

Polyethylene glycol fatty acid esters
Polyglycerine fatty acid esters

- 25 Preferably, the nonionic surfactant is HLB 9 or more and non-toxic to human body. More preferable one is polyoxyethylene sorbitan fatty acid esters.

The surfactant and the compound (I) in the suspendible composition of the present invention is preferably in the ratio of 0.01 : 1 to 5 : 1 by weight, more preferably 0.1 : 1 to 1 : 1 by weight.

- 30 The concentration of the tricyclic compound (I) in the suspendible composition of the present invention is not specifically limited, but is preferably 0.001 to 2 wt.%, more preferably 0.01 to 1 wt.% in the suspension to which an aqueous medium is added. The concentration of the surfactant varies with type, but generally 0.001 to 1 wt.%, more preferably 0.005 to 0.2 wt.% in the suspension to which the aqueous medium added.

- 35 If desired, the suspendible composition of the present invention may contain conventional additives which are used in solutions or eye drops, such as anticoagulating agents (e.g., hydroxypropylmethylcellulose, D-mannitol or the like); anti-foaming agents (e.g., silicones such as dimethicone); isotonic agents (e.g., sodium chloride or the like); buffer agents (e.g., boric acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, or the like); preservatives (e.g., benzalkonium chloride, benzethonium chloride, chlorobutanol, methylparaben, propylparaben or the like); thickening agents (e.g., saccharides such as lactose, mannitol, maltose or the like, hyaluronic acid or its salt such as sodium hyaluronate, potassium hyaluronate or the like, mucopolysaccharides such as chondroitin sulfate or the like, sodium polyacrylate, carboxyvinylpolymer, cross-linked polyacrylate or the like) and other additives.

- 40 The suspendible composition of the present invention can be prepared by adding, to the fine particle (average particle size: 5 μ m or below) of the tricyclic compound (I) or its salt or the lyophilized product obtained as mentioned above, the surfactant or its aqueous solution (the latter is used if required when the tricyclic compound (I) is in the form of lyophilized product), and optionally additives such as anticoagulating agent, antifoaming agent, isotonic agent, buffer agent, preservative and/or thickening agent in a suitable amount, the resultant being then well mixed.

- 45 Preferably, the tricyclic compound (I) or its salt is in the form of a composition which can be used as an aqueous suspension by addition of an aqueous medium in situ from the viewpoint of stability of the tricyclic compound (I).

- 50 The therapeutically effective dose of the tricyclic compound (I) or its salt varies with age and kinds or level of diseases of each patient, but generally 0.01 to 1000 mg per day, preferably 0.1 to 500 mg per day, and more preferably 0.5 to 100 mg per day. Usually, the tricyclic compound (I) or its salt is administered in an average amount of 0.5 mg, 1 mg, 5 mg, 10 mg, 50 mg, 100 mg, 250 mg, and 500 mg per day.

- 55 The eye drops including the tricyclic compound (I) or its salt is usually applied to an eye in 2 to 3 drops, one to several times per day.

The suspendible composition of the present invention can also be prepared by employing the compounds disclosed in the following publications such as EP-A-353678, Japanese Patent Application No. HEI 2(1990)-74330, PCT/GB90/01262, EP-A-413532, PCT/JP91/00314, British Patent Application No. 9012963.6, British Patent Application No. 9014136.7, British Patent Application No. 9014681.2, British Patent Application No. 9014880.0, British Patent Application No. 9014881.8, British Patent Application No. 9015098.8, British Patent Application No. 9016115.9, British Patent Application No. 9016693.5, EP-A-323865, EP-A-349061, EP-A-358508, EP-A-364031, EP-A-364032, EP-A-378317, EP-A-378320, EP-A-378321, EP-A-388153, EP-A-396399, EP-A-396400, EP-A-399579, EP-A-403242, EP-A-428365, EP-A-356399, GB 2225576 A, EP-A-402931 and EP-A-427680.

The present invention will be explained hereinbelow with reference to the Examples.

Example 1

Preparation of Fine Particles of FK 506 Substance

FK 506 substance (3 g) was dissolved in ethanol (15 ml), to which 10 % aqueous hardened polyoxyethylene castor oil solution (HCO-60®: manufactured by Nikko Chemicals Co., Ltd.) or 10 % aqueous monooleic polyoxyethylene sorbitan 20EO solution (polysorbate 80, Reodol TWO-120®: manufactured by Kao-Atras Co., Ltd.) (6 ml each) was added dropwise under stirring. Thereafter, distilled water (9 ml) was dropwise added in a small amount under slow stirring at 25 °C. Further, distilled water (80 ml) was added under rapid stirring and further stirred for about 15 minutes. Finally, distilled water (60 ml) was added under rapid stirring to precipitate crystals of FK 506 substance which was allowed to stand overnight at 25 °C. The suspension thus obtained was subjected to ultrasonic wave treatment for 2 minutes to disperse and filtered through a filter (Millipore Co., Ltd.) having a pore size of 0.45 µm under reduced pressure to obtain wet crystals (average particle size: 0.9 µm) of FK 506 substance.

Fine particles of various particle sizes were prepared by the same manner as described above.

Subsequently, prescription examples of the present invention will be explained.

Prescription 1

FK 506 substance (average particle size: 0.8 µm)	1.0 %
Polyoxyl stearate 40 (dispersing agent)	0.05 %
Benzalkonium chloride (preservative)	0.02 %
Sodium chloride (isotonic agent)	0.288%
Phosphate buffer (pH 4.5) q.s.	to 100

Each component was mixed according to a known method to afford aqueous suspendible eye drops.

Aqueous suspensions for eye drops of the Prescription 2 to Prescription 6 were prepared by the same manner as in Prescription 1.

Prescription 2

FK 506 substance (average particle size: 3.0 µm)	0.5 %
Polysorbate 80 (dispersing agent)	0.05 %
Polyvinyl alcohol (dispersing agent)	0.28 %
Chlorobutanol (preservatives)	0.5 %
Glycerine (isotonic agent)	2.47 %
Phosphate buffer (pH 4.5) q.s.	to 100

EP 0 484 936 A1

Prescription 3

5

10

FK 506 substance (average particle size:2.0 μm)	0.2 %
Polyoxyethylene polyoxypropylene ethyl ether (dispersing agent)	0.1 %
Polyvinylpyrrolidone (dispersing agent)	0.06 %
Methylparaben (preservatives)	0.04 %
Propylparaben (preservatives)	0.02 %
Glycerine (isotonic agent)	2.47 %
Phosphate buffer (pH 5.0) q.s.	to 100

Prescription 4

15

20

FK 506 substance (average particle size:1.5 μm)	0.1 %
Polysorbate 80	0.05 %
Polyvinylalcohol	0.28 %
Benzalkonium chloride	0.02 %
Sodium chloride	0.280%
Phosphate buffer (pH 4.5) q.s.	to 100

25

Prescription 5

30

FK 506 substance (average particle size:0.9 μm)	0.1 %
Polysorbate 80	0.05 %
Benzalkonium chloride	0.02 %
Phosphate buffer (pH 4.5) q.s.	to 100

35

Prescription 6

40

45

FK 506 substance (average particle size:0.9 μm)	0.1 %
Polysorbate 80	0.05 %
Benzalkonium chloride	0.02 %
Sodium chloride	0.288%
Phosphate buffer (pH 4.5) q.s.	to 100

50

55

Prescription 7

FK 506 substance (average particle size:1.0 μ m)	1.0 mg
Polysorbate 80	1.0 mg
Polyvinylalcohol	2.8 mg
Methylparaben	0.8 mg
Propylparaben	0.2 mg
Citric acid	5.0 mg
Sodium hydroxide	suitable amount for adjusting pH 4.5
Purified water q.s.	to 1.0 ml

Each component was mixed by a conventional method to prepare an oral aqueous suspension.

Prescription 8Suspendible Composition

FK 506 substance (average particle size 1.0 μ m)	50 mg
Polysorbate 80	25 mg
Hydroxypropylmethylcellulose (TC-5S)	150 mg
Simethicone (i.e., dimethyl polysiloxance, silicon anti-foaming agent)	2 mg
D-Mannitol	250 mg

Dispersing Medium

Methylparaben	40 mg
Propylparaben	10 mg
Purified water q.s.	to 50 ml

A solution of polysorbate 80 in purified water was added to a solution of FK 506 substance in ethanol followed by well mixing. To the resultant was gradually added an appropriate amount of water to crystallize out FK 506 substance. The resultant mixture was aged, concentrated and subjected to ultrasonic wave treatment. The mixture was mixed and suspended in a solution of hydroxypropylmethylcellulose and D-mannitol in purified water. The resultant was poured into a vessel filled with simethicone and then lyophilized to afford the suspendible composition.

On the other hand, methylparaben and propylparaben were dissolved in purified water to afford the dispersing medium. An aqueous suspension can be prepared by adding the aforesaid dispersing medium to the aforesaid suspendible composition, if required.

The effect of the present invention will be explained hereinbelow with reference to Test Examples.

1. Absorption Test by applying eye drops

Eye drop to be tested

Eye drop (1) obtained in Prescription 5

Test method

The test eye drop was applied five times to both eyes of 8 - 10-week old male SD-rats (weight: 275 to 405 g) in an amount of 10 μ l at an interval of 5 minutes. After an hour, the rats were bled to death for extracting both eyes. The concentration of FK 506 substance at the cornea and retina and choroid of the extracted eyes was measured in accordance with a known enzyme immunoassay (e.g., indirect method disclosed in Japanese Laid-Open Patent Publication No. HEI 1-92659).

The results were shown in Tables 1 and 2. The concentration was represented by mean \pm standard error of mean.

Results

Table 1 (concentration of FK 506 substance at cornea)

Eye Drop	Conc. of FK 506 substance [ng/tissue wt. (g)]
	After an hour
Eye Drop (1)	374.3 \pm 110.9

Table 2 (conc. of FK 506 substance at retina and choroid)

Eye Drop	Conc. of FK 506 substance [ng/tissue wt. (g)]
	After an hour
Eye Drop (1)	22.5 \pm 1.7

2. Absorption Test by oral administration

Oral agent to be tested

The aqueous suspension obtained in Prescription 7 was used.

Test Method

The oral agent mentioned above was diluted with purified water to have the concentration of FK 506 substance of 0.064 mg/ml. The resultant was orally administered to six cynomolgus monkeys (0.32 mg/kg).

The concentration of FK 506 substance in whole blood was measured 0.5, 1, 2, 4 and 6 hours after the administration in accordance with the aforesaid enzyme immunoassay.

The results are shown in Table 3. The test results are represented by the average \pm standard error.

Table 3

(conc. of FK 506 substance in whole blood)	
Time	Conc. in whole blood (ng/ml)
0.5	13.38 \pm 9.88
1	18.65 \pm 9.36
2	18.63 \pm 9.89
4	15.33 \pm 7.77
6	12.45 \pm 9.33

As apparent from the above, the suspendible composition of the present invention can provide a formulation having excellent penetration into internal tissues, especially a formulation exhibiting excellent penetration into internal tissues through oral administration and ocular tissues through application to an eye.

Accordingly, the present invention can provide a formulation of the tricyclic compound (I) which is easy to adjust the administration amount, is excellent in stability, dispersibility and penetration into ocular tissues, is capable of applying to eye and is easy to administer to children. The formulation of the present invention can also aspirate.

In view of pharmacological activities of the tricyclic compound (I) such as immunosuppressive activity, antimicrobial activity, and the like, the formulation of the present invention is useful for the treatment and prevention of immune-mediated diseases such as the resistance by transplantation of organs or tissue such as heart, kidney, liver, medulla ossium, skin, cornea, lung, pancreas, intestinum tenue, limb, muscle, nervus, etc.; graft-versus-host diseases by medulla ossium transplantation; autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type I diabetes, and the like; and further infectious diseases caused by pathogenic microorganisms.

Further, the formulation of the present invention is useful for the treatment and the prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as, psoriasis, atopic dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata;

various eye diseases such as autoimmune diseases and so on (e.g. keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's disease, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leukoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' ophthalmopathy, Vogt-Koyanagi-Harada syndrome, sarcoidosis, etc.);

reversible obstructive airways disease, which includes conditions such as asthma (e.g., bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (e.g., late asthma and airway hyper-responsiveness), bronchitis and the like; inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis, ischemic bowel disease, inflammatory bowel disease, necrotizing enterocolitis, intestinal lesions associated with thermal burns, leukotriene B₄-mediated diseases;

intestinal inflammations/allergies such as Coeliac disease, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis;

food related allergic diseases which have symptomatic manifestation remote from the gastro-intestinal tract, for example, migraine, rhinitis and eczema;

renal diseases such as interstitial nephritis, Goodpasture's syndrome, hemolytic-uremic syndrome and diabetic nephropathy;

nervous diseases such as multiple myositis, Guillain-Barré syndrome, Ménière's disease and radiculopathy;

endocrine diseases such as hyperthyroidism and Basedow's disease;

hematic diseases such as pure red cell aplasia, aplastic anemia, hypoplastic anemia, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, agranulocytosis and anerythroplasia;

bone diseases such as osteoporosis;

respiratory diseases such as sarcoidosis, fibroid lung and idiopathic interstitial pneumonia;

skin diseases such as dermatomyositis, leukoderma vulgaris, ichthyosis vulgaris, photoallergic sensitivity and cutaneous T cell lymphoma;

circulatory diseases such as arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa and myocardosis;

collagen diseases such as scleroderma, Wegener's granuloma and Sjogren's syndrome;
adiposis;

eosinophilic fasciitis;

periodontal disease such as lesion of gingiva, periodontium, alveolar bone, substantia ossea dentis;

5 nephrotic syndrome such as glomerulonephritis;

male pattern alopecia or alopecia senilis;

muscular dystrophy;

Pyoderma and Sezary's syndrome;

10 active oxgen-mediated diseases, for example, organ injury such as ischemia-reperfusion injury of organs (e.g., heart, liver, kidney, digestive tract) which occurs on preservation, transplantation or ischemic diseases (e.g., thrombosis, cardiac infarction); intestinal diseases such as endotoxin-shock, pseudomembranous colitis, colitis caused by drug or radiation; renal diseases such as ischemic acute renal insufficiency, chronic renal insufficiency; pulmonary diseases such as toxinoses caused by lung-oxygen or drug (e.g., paracort, bleomycins), lung cancer, pulmonary emphysema; ocular diseases such as cataracta, siderosis, retinitis, 15 pigmentosa, senile macular degeneration, vitreal scarring, corneal alkali burn; dermatitis such as erythema multiforme, linear IgA ballous dermatitis, cement dermatitis; and others such as gingivitis, periodontitis, sepsis, pancreatitis, diseases caused by environmental pollution (e.g., air pollution), aging, carcinogenesis, metastasis of carcinoma, hypobaropathy;

20 diseases caused by histamine or leukotriene C₄ release; and so on.

Further, the active ingredient of the present invention has liver regenerating activity and/or activities of stimulating hypertrophy and hyperplasia of hepatocytes. Therefore, the formulation of the present invention is useful for the treatment and prevention of hepatic diseases such as immunogenic diseases (e.g., chronic autoimmune liver diseases such as the group consisting of autoimmune hepatitis, primary biliary cirrhosis and sclerosing cholangitis), partial liver resection, acute liver necrosis (e.g., necrosis caused by toxins, viral 25 hepatitis, shock or anoxia), B-virus hepatitis, non-A/non-B hepatitis, cirrhosis and hepatic failure such as fulminant hepatic failure, late-onset hepatic failure and "acute-on-chronic" liver failure (acute liver failure on chronic liver diseases).

Moreover, the formulation of the present invention is useful for various diseases because of its useful pharmaceutical activity such as augmenting activity of chemotherapeutic effect, preventing or treating 30 activity of cytomegalovirus infection, anti-inflammatory activity, and so on.

Claims

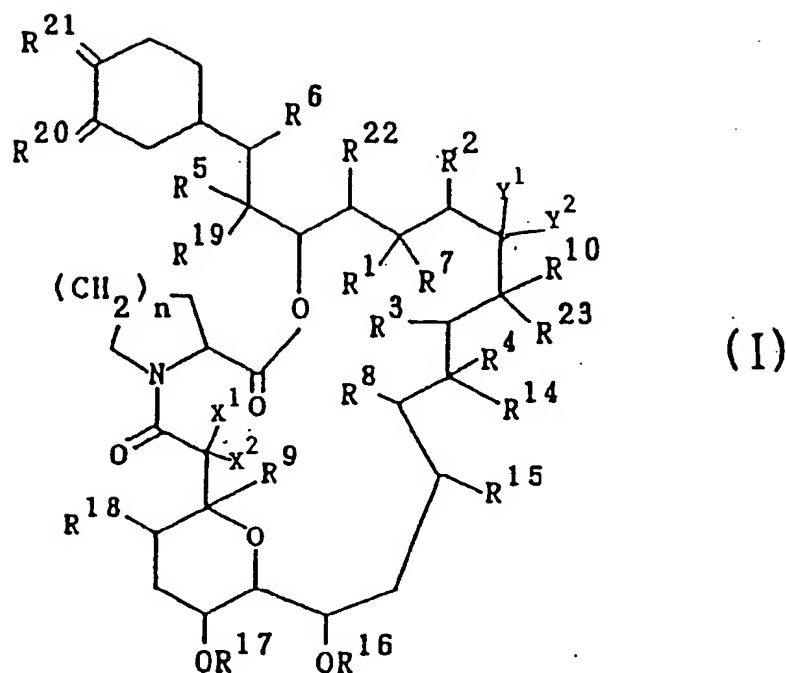
- 35 1. A suspendible composition comprising fine particles of a tricyclic compound represented by the following formula (I):

40

45

50

55



wherein each of adjacent pairs of R¹ and R², R³ and R⁴ or R⁵ and R⁶ independently

(a) is two adjacent hydrogen atoms, or

(b) may form another bond formed between the carbon atoms to which they are attached, and further, R² is an alkyl group;

R⁷ is a hydrogen atom, a hydroxy group, a protected hydroxy group or an alkoxy group, or an oxo group together with R¹;

R⁸ and R⁹ each is independently a hydrogen atom or a hydroxy group;

R¹⁰ is a hydrogen atom, an alkyl group, an alkyl group substituted by one or more hydroxy groups, an alkenyl group, an alkenyl group substituted by one or more hydroxy groups or an alkyl group substituted by an oxo group;

X¹ is a hydrogen atom or a hydroxy group;

X² is a hydrogen atom; or

X¹ and X² may together represent an oxo group or -CH₂O-;

Y¹ is a hydrogen atom or a hydroxy group;

Y² is a hydrogen atom; or

Y¹ and Y² may together represent an oxo group, N-NR¹¹R¹² or N-OR¹³;

R¹¹ and R¹² each is independently a hydrogen atom, an alkyl group, an aryl group or a tosyl group;

R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²² and R²³ each is independently a hydrogen atom or an alkyl group;

R²⁰ and R²¹ each is an oxo group or independently (R²⁰a and a hydrogen atom) or (R²¹a and a hydrogen atom) in which R²⁰a and R²¹a each is independently a hydroxy group, an alkoxy group or a group represented by the formula -OCH₂OCH₂CH₂OCH₃, or R²¹a is a protected hydroxy group, or R²⁰a and R²¹a may together represent an oxygen atom in an epoxide ring;

n is an integer of 1, 2 or 3;

in addition to their above definitions, four of Y¹, Y², R¹⁰ and R²³, together with the carbon atoms to which they are attached, may represent a saturated or unsaturated 5- or 6-membered nitrogen, sulfur and/or oxygen containing heterocyclic ring optionally substituted by one or more groups selected from the group consisting of an alkyl, a hydroxy, an alkyl substituted by one or more hydroxy, an alkoxy, benzyl and a group of the formula -CH₂Se(C₆H₅);

or a pharmaceutically acceptable salt thereof, said fine particles of the tricyclic compound and its pharmaceutically acceptable salt having the average size of 5 μm or below;

a pharmaceutically acceptable surfactant; and optionally an aqueous medium.

2. A suspendible composition as claimed in claim 1 in which the pharmaceutically acceptable surfactant is a nonionic surfactant.
3. A suspendible composition as claimed in claim 2 in which the nonionic surfactant is polyoxyethylenesorbitan fatty acid ester.
4. A suspendible composition as claimed in claim 3 which is in the form of aqueous eye drops.
5. A suspendible composition as claimed in claims 1 to 4 in which the tricyclic compound (I) is the one wherein each of adjacent pairs of R³ and R⁴ or R⁵ and R⁶ independently may form another bond formed between the carbon atoms to which they are attached, R⁸ and R²³ each is independently a hydrogen atom; R⁹ is a hydroxy group; R¹⁰ is methyl, ethyl, propyl or allyl; X¹ is a hydrogen atom; X² is a hydrogen atom; or X¹ and X² may together represent an oxo group; Y¹ and Y² may together represent an oxo group; R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹ and R²² each is independently methyl; R²⁰ and R²¹ independently are (R^{20a} and a hydrogen atom) or (R^{21a} and a hydrogen atom) in which R^{20a} and R^{21a} each is a hydroxy group or an alkoxy group or R^{21a} is a protected hydroxy group; and n is an integer of 1 or 2.
6. A suspendible composition as claimed in claims 1 to 5 in which the tricyclic compound (I) is the one wherein R⁷ is a hydrogen atom, a hydroxy group or a protected hydroxy group; X¹ and X² may together represent an oxo group; R^{20a} is methoxy; and R^{21a} is a hydroxy or protected hydroxy group.
7. A suspendible composition as claimed in claims 1 to 6 in which the tricyclic compound (I) is 17-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]-octacos-18-ene-2,3,10,16-tetraone or 17-ethyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]-octacos-18-ene-2,3,10,16-tetraone.
8. A process for preparing a suspendible composition which comprises
 - dissolving the compound (I) in claim 1 or its pharmaceutically acceptable salt in an organic solvent which can dissolve it;
 - mixing the resultant solution with an aqueous solution of a pharmaceutically acceptable surfactant;
 - adding water to the resultant mixture to crystallize out the tricyclic compound (I) or its pharmaceutically acceptable salt;
 - if desired, subjecting the mixture containing the crystals to aging, concentration and micronization to obtain fine particles having an average particle size of 5 µm or below, lyophilizing the fine particles to afford a lyophilized product; and
 - further if desired, adding an aqueous medium to the lyophilized product.
9. A process for preparing a suspendible composition which comprises mixing the fine particles having an average particle size of 5 µm or below of the tricyclic compound (I) in claim 1 or its pharmaceutically acceptable salt with a pharmaceutically acceptable surfactant, and if desired adding an aqueous medium to the resultant mixture.
10. Use of the tricyclic compound of the formula (I) as defined in claim 1 or its pharmaceutically acceptable salt in the preparation of a suspendible composition containing at least one pharmaceutically acceptable surfactant.



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 11 8982

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,A	WO-A-8 905 304 (FISONS) * Claims 1,8-9; page 16, lines 24-25; page 17, lines 1-20, in particular lines 11-14 *	1,5-8, 10	A 61 K 9/06 A 61 K 31/44 A 61 K 31/445 A 61 K 31/55 A 61 K 47/26
A	EP-A-0 406 791 (FUJISAWA) * Claims *	1,4-10	
D,A	EP-A-0 184 162 (FUJISAWA) * Claims 1,15-20; page 76, lines 10-34 *	1,5-7, 10	
P,A	EP-A-0 428 169 (FUJISAWA) * Claims; page 5, lines 9-28 *	1-3,5-8, 10	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			A 61 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 09-01-1992	Examiner SCARPONI U.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPO FORM 1503 01.82 (P0401)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER: _____**

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.